# 4. RADIAL FIVEFOLD ROTATION: A NEW METHOD IN THE STUDY OF THE BIOPOLYMER ORGANIZATION OF THE SPORODERM

M. KEDVES, A. TÓTH and A. VÉR

*Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany of the J. A. University, H–6701, Szeged, Hungary*

## Abstract

The two major constituents, the quasi-crystalloid skeleton and its stabilizing molecular system were established previously on partially degraded sporoderms. A peculiar and heterogeneous biopolymer skeleton was discovered in the wall of *Botryococcus braunii* KÜTZ. isolated from a Hungarian oil shale. Quasi-periodic and quasi-equivalent symmetry was observed on the different levels of organization of the biopolymer system of this species. The new method was elaborated on the peculiar quasi-periodic units of the biopolymer skeleton. Two kinds of radial rotations methods were elaborated for the first time. One with a positive, the other with a negative secondary points of symmetry centre. The rotation area was introduced as a new methodical term. Single and combined, inside of the combined outest and innermost summarized areas were distinguished. In some cases secular alterations in the size and shape of the rotation areas were established.

*Key words: Botryococcus braunii,* oil shale, biopolymer symmetry, new method.

## Introduction

The peculiar algue, *Botryococcus braunii* KÜTZ. has been the subject of several investigations, and a number of papers have appeared in different aspects of this subject. The result of the LM studies have been summarized recently by VÉR (1994). The combined investigations of the Transdanubian oil shale in Hungary was reviewed in this paper and will be reviewed in further ones. TEM results of intact colonies were published by KEDVES (1983), and later by GLIKSON, LINDSAY and SAXBY (1989). Experimental investigations were carried out with different methods (cf. KEDVES, 1986, KEDVES, ROJIK and VÉR, 1991, KEDVES, TÓTH and FARKAS, 1993). The peculiarities of the biopolymer structure were also established particularly on the partially degraded and fragmented wall of the *Botryococcus* colonies isolated from the Hungarian oil shale. As the most important peculiarity, we can emphasize that the quasi-periodic and quasi-equivalent biopolymer organization is present in the wall of the colonies of *Botryococcus braunii.*

As it has been emphasized in several papers, one of the research programs of our laboratory is the investigation of the symmetry and the organization of the biopolymer system of different kinds of plant cell walls. The most important fields of research are the following:

1. Investigation of the quasi-crystalloid biopolymer skeleton.
2. Investigation of the symmetry and organization of the stabilizing biopolymer system of quasi-crystalloid skeleton.
3. On the biopolymer and molecular level it is necessary to pay special attention to the outer and inner bordering surfaces.

Modellings are very important in the investigation of the biopolymer structure of the plant cell wall. We have used the following methods so far:

1. Two-dimensional modelling with the modified MARKHAM rotation method (cf. KEDVES, 1988, 1989, 1990, 1991a, KEDVES and FARKAS, 1991, KEDVES, PÁRDUTZ, FARKAS and VÉR, 1991, KEDVES, ROJIK and VÉR, 1991, etc.).

2. Three-dimensional modelling of the quasi-crystalloid skeleton (KEDVES, 1991b, 1992, KEDVES, TÓTH and FARKAS, 1993).

3. Computer modelling of the quasi-crystalloid skeleton and the stabilizing system (cf. M. KEDVES and L. KEDVES, 1995, in this number).

The aim of this paper is to complete the opportunities of two-dimensional rotation, essentially with the modified MARKHAM rotation method. This method was elaborated for the peculiar biopolymer system of the *Botryococcus braunii* KÜTZ., but it seems to be useful for other quasi-crystalloid biopolymer structures, as well.

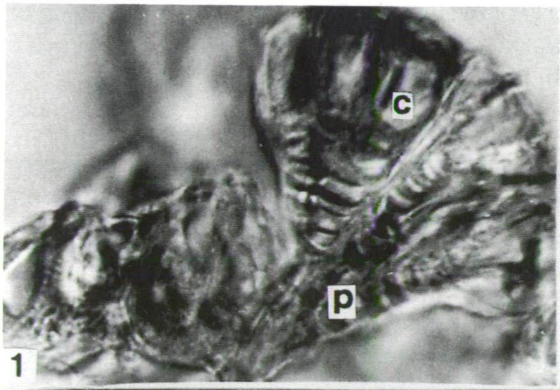
## Materials and Methods

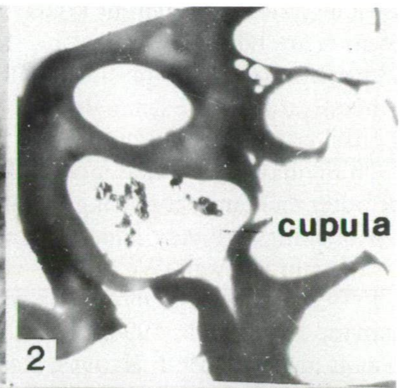
The material of investigation is identical with those published previously (KEDVES, TÓTH and FARKAS, 1993, p. 41). In the paper mentioned above the basic biopolymer pentagons of number I, II, and III were used for biopolymer symmetry operations. Now further biopolymers, designated with IV and V were the subjects of our studies (Plate 4.1., fig. 3). The description of the method was published in our paper (KEDVES, TÓTH and FARKAS, 1993), p. 41, as follows: "*Botryococcus braunii* KÜTZ., from the Upper Tertiary Oil Shale, Pula, Hungary. (Experiment No. 924). Ten mg of air dried colonies was added to one ml of merkaptoethanol. A temperature of 30 °C was maintained for 24 hours. The residue was washed and fragmented with a magnetic stirrer in an aqueous medium for 30 minutes. The fragmented wall remnants were dropped onto a grid, covered with a collodium pellicule and then dried. The EM investigations were made on a Tesla BS-500 TEM (resolution 6 Å) at the J. A. University, Faculty of Science, Electron Microscope Laboratory."

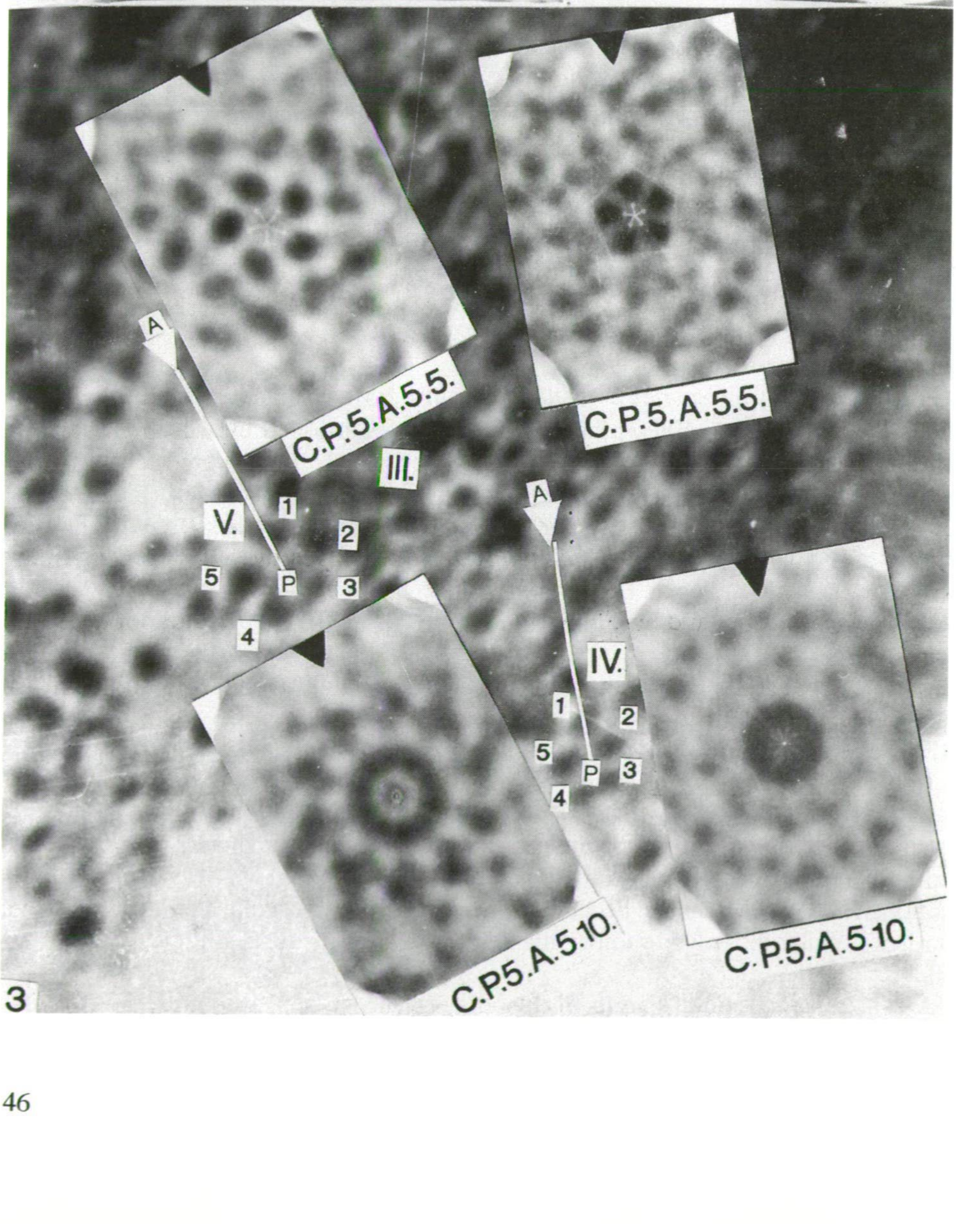
Concerning the biopolymer units investigated, biopolymer no IV is a so-called "normal" regular pentagonal biopolymer unit.

Biopolymer no V is peculiar, because in the middle of the regular pentagon there is another globular biopolymer unit. This latter was used for the first radial rotation as follows:

A) The ten dark globular units of the pentagon which appeared after the fivefold rotation C.P.5.A.5.5.


c
p
1


cupula
2


C.P.5.A.5.5.
C.P.5.A.5.5.
III.
A
V.
1
2
5
P
3
4
A
IV.
1
2
5
P
3
4
C.P.5.A.5.10.
C.P.5.A.5.10.
3

B) The ten light points of symmetry (holes of biopolymer dimension) of the tenfold rotation (C.P.5.A.5.10.).

## Results

The recently elaborated radial rotation is always a kind of secondary rotation, and it is two-dimensional.

The secondary points of symmetry of a fivefold or tenfold primary rotation are used as rotation centres.

The axes are linear between the centre (P) of the basic biopolymer unit of the primary rotation and one of the secondary point of symmetry.

The centrum of the radial rotation is always a secondary point of symmetry.

The centrum of the radial secondary rotation can be positive or negative; one globular biopolymer unit,

or a hole of the dissolved exine.

Designations; for example C.S.5.R.$_{1+1/10}$.5.5.

C = complete rotation – the total of the angles of rotation is 360°.

S = secondary rotation – the centre of rotation is one of the new points of symmetry which appeared after a primary rotation.

5 = fivefold rotation.

R = radial rotation; see above.

1+1/1-10 = the first circle, +1 first positive, dark points of symmetry, 1-10 – ten secondary biopolymer units were radially rotated.

5.5. = number of exposition – 5 x 5.

C.S.5.5.$_{1\text{-}1/1\text{-}10}$.5.5. indicates the tenfold radial rotation of the ten light points of symmetry (holes). There is one difference only in the designation, 1-1/1-10 indicates that the points of symmetry of the centres of the radial rotation are light (holes of one biopolymer unit).

The common results of the two kinds of rotations are the following:

1. In both cases the secondary radial rotations resulted in positive and negative points of symmetry.
2. Characteristic outer and inner areas can be established after the radial rotations.

◄ Plate 4.1.

1. LM picture of *Botryococcus braunii* KÜTZ. isolated from Hungarian oil shale, slide: Pula 1/1, cross-table number: 9.3/115.1. c = cupula, p = pedunculus. 1000x.
2. TEM picture from non-experimental colony. The compact substance of the wall is well illustrated. 8.000x.
3. Detail of a partially degraded colony. Experiment No: 924, negative no: 586. The basic pentagonal biopolymer units designated with IV and V were the subject of fivefold and tenfold rotations. The basic TEM picture, the investigated biopolymer units with the PA rotation axes, and the rotation pictures are illustrated here. 500.000x.

3. The points of symmetry of the outest and innermost areal borders may be useful for further biopolymer symmetry operations.
4. The summarized border is composed from the outest limits of the outest areas.
5. There are points of symmetry outside of this summarized border. Its origin is interesting and doubtful; using these points of symmetry as rotation centrums, we may hope for interesting results.

Regarding the details, we can point out the following:

## RADIAL ROTATION WITH THE POSITIVE POINTS OF SYMMETRY IN THE CENTRUM

(Plate 4.2., text-fig. 4.1., 4.2., 4.3.)

At the inner and the outer rotation areas several periodicities may be established.

The outer rotation areas. – The summarized outer areas indicate that there are several irregular inner fields which overlap one another, resulting in dark fields (Text-fig. 4.1.). The drawings were made with altered perpendicular and horizontal streakings. (The first one is horizontal, the second one is perpendicular, the third one is horizontal again). The contour of the overlapping areas – the darkest area is irregular in form.

Inner rotation areas. – The smaller, inner rotation areas overlap very poorly, they can be seen relatively easily in their contour. In this case the following similarities can be established: In some cases the opponent inner fields may be identical or more or less similar; areas no 2 and 7 are the best examples in this respect (Text-fig. 4.2.). As contrary examples for the inner area no 5 and 10 can be pointed out. The greatest number of the inner areas are more or less identical, cf. 1–6. The border of the summarizing outer areas with the so-called extra-areal points of symmetry are represented on Text-fig. 4.2. The disposition of the points of symmetry following the origin between the rotation axes is the following.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 – 2 | 2 | 7 | | | | | | | | |
| 2 – 3 | | 2 | 3 | 1 | | | | | | |
| 3 – 4 | | | 3 | 5 | | | | | | |
| 4 – 5 | | | | 5 | 6 | 2 | | | | |
| 5 – 6 | | | | 4 | 8 | | | | | |
| 6 – 7 | | | | | | 11 | 6 | 2 | | |
| 7 – 8 | | | | | | 1 | 4 | 6 | | |
| 8 – 9 | | | | | | | 1 | 10 | 8 | |
| 9 – 10 | | | | | | | | | 6 | 9 |
| 10 – 1 | 3 | 1 | | | | | | | | 7 |

Some points of symmetry are on the axes. The different kinds of rotation of these extra-areal points of symmetry will make another interesting methodical problem.

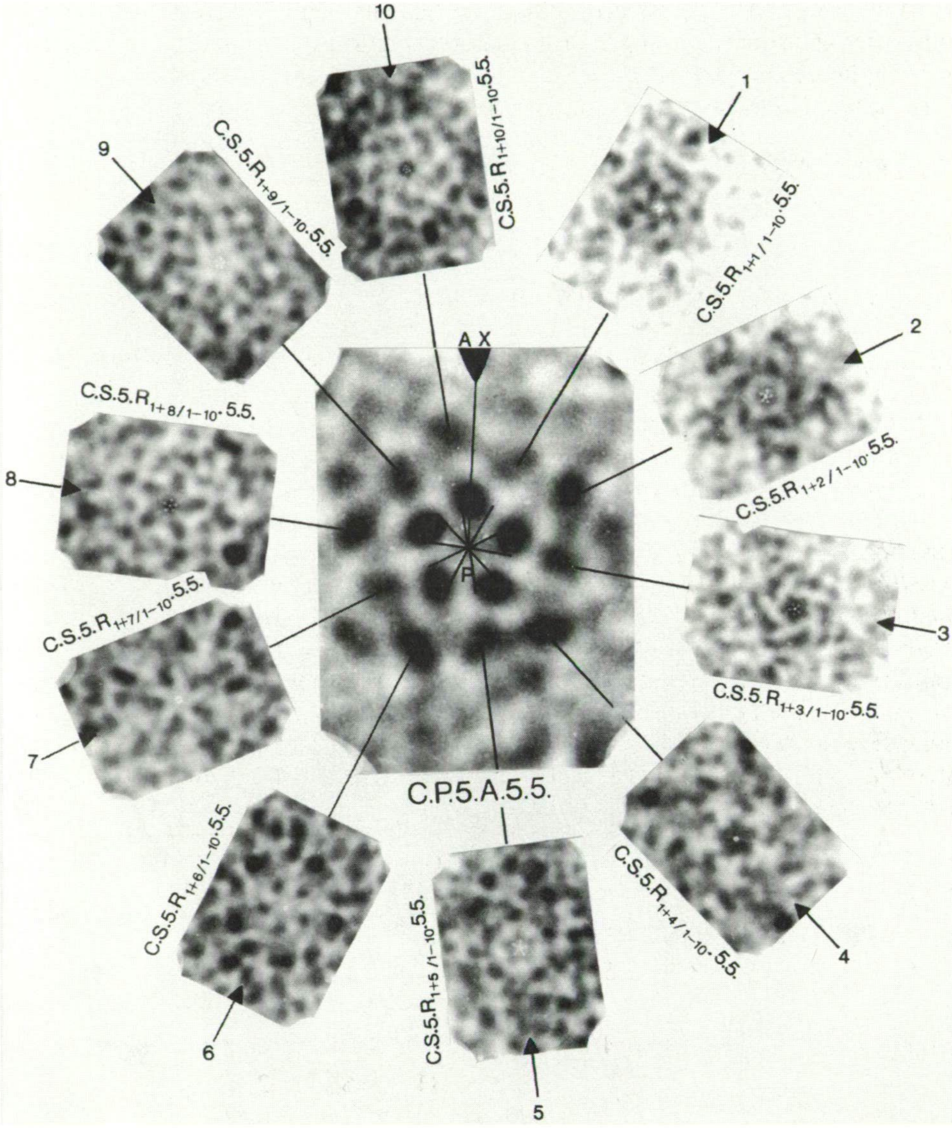
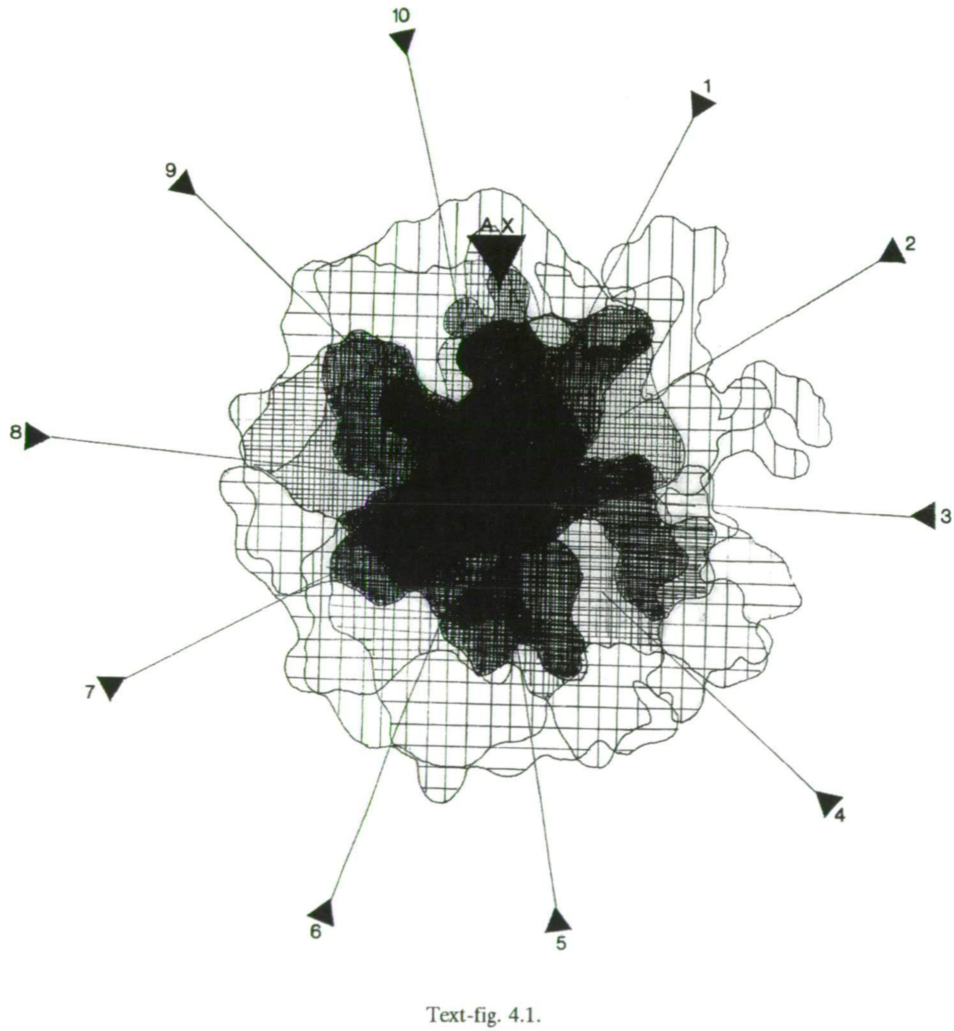


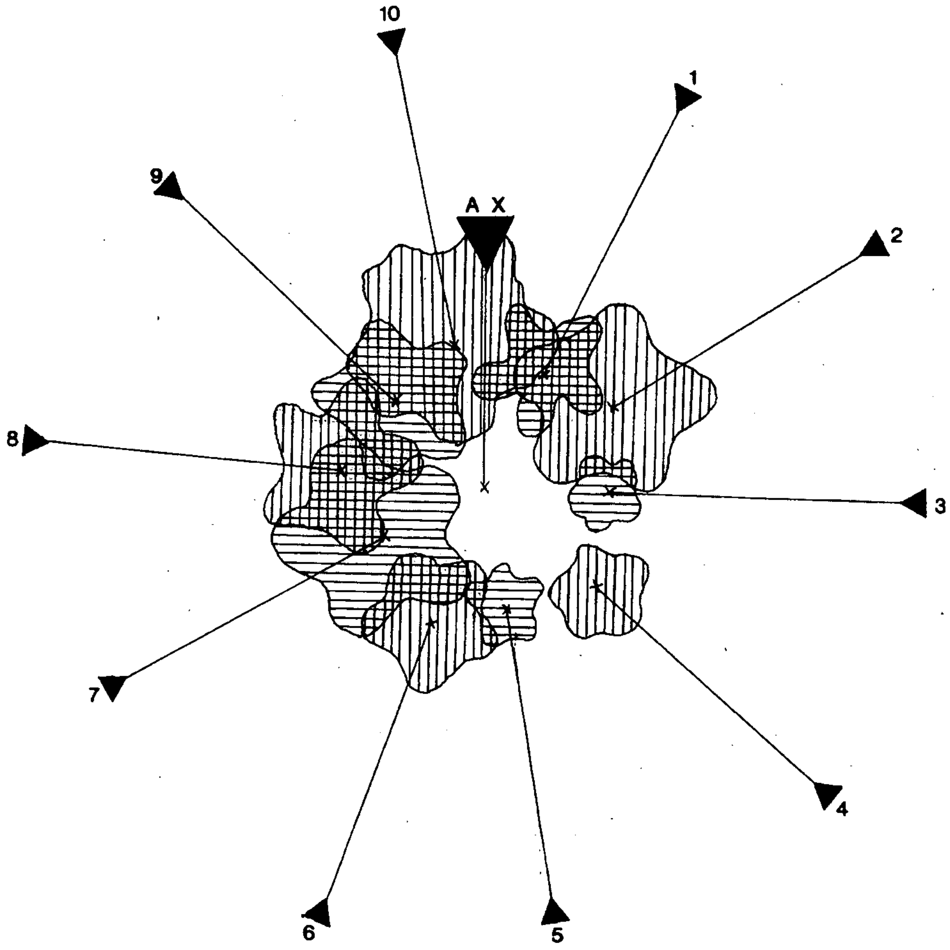

Plate 4.2.

The fivefold rotation picture of the basic biopolymer unit no V as a basis for positive radial rotation. The ten rotation centres are the positive points of symmetry which are surrounding the basic pentagon. The pictures of the radial rotation are placed in the direction of the axes of the radial rotation.
$R_{1\ +1/1\text{-}10} - R_{1\ +10/1\text{-}10}$



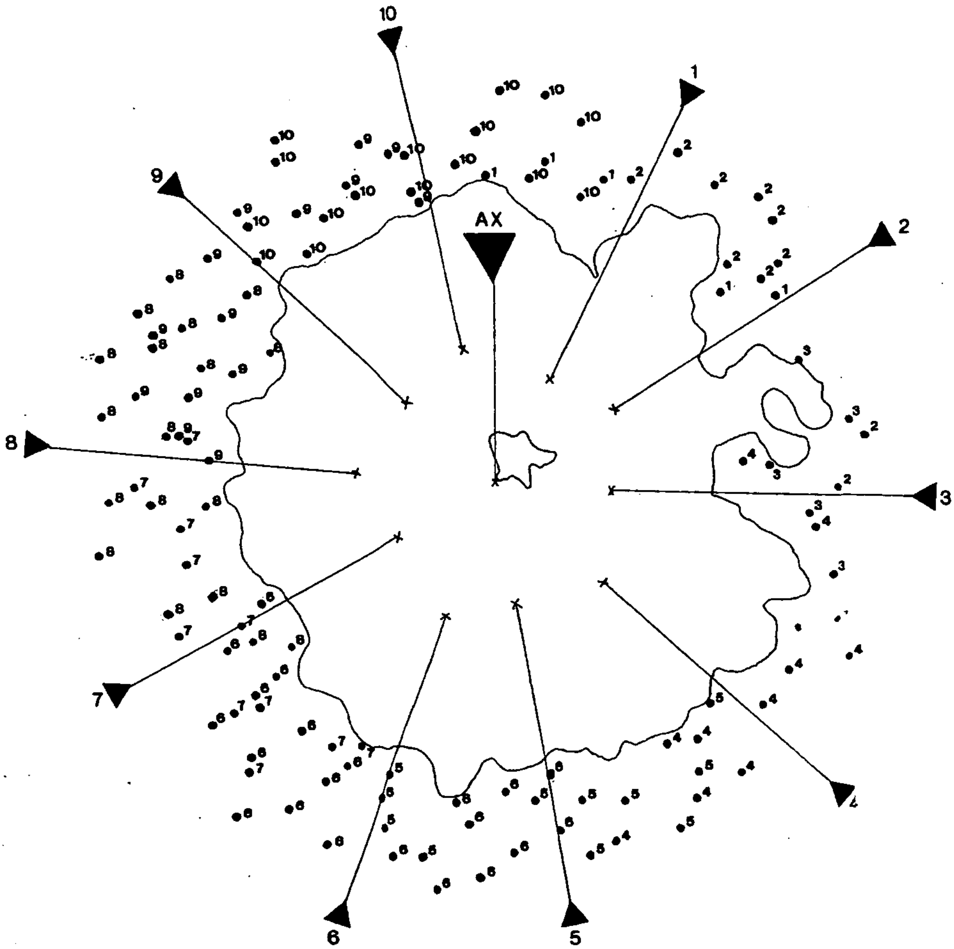

Text-fig. 4.1.

The outer rotation areas of the ten positive radial rotations, cf. Plate 4.2.




Text-fig. 4.2.

The inner rotation areas of the ten positive radial rotations, cf. Plate 4.2. A certain periodicity may be established in the shape and size of some of the opposite inner rotation areas (1–6, 2–7), these are more or less identical. 3–8, 4–9, 5–10 are similar in contour, but the size of the rotation areas of the lower values (3, 4, 5) is much smaller than those of the higher ones (8, 9, 10).




Text-fig. 4.3.

The summarized outer and inner borders of the positive rotation areas with axes of rotations, and the extra-areal points of symmetry. The numbers of the extra-areal points of symmetry indicate the "origin" of these points of symmetry, e. g.: the number of the radial rotations.

## RADIAL ROTATION WITH NEGATIVE POINTS OF SYMMETRY IN THE CENTRUM

(Plate 4.3., text-fig. 4.4., 4.5., 4.6.)

The outer rotation areas. – The summarized outer areas in this case are more or less regular than previously. One dark centrum appears rounded with by and by lighter concentric zones (Text-fig. 4.4.). On the contour of the outest summarized border a certain asymmetry may be established.
Inner rotation areas (Text-fig. 4.5.). – In this case it is not so easy to establish similarities or differences between the opposite areas. The summarized outer border of these areas is more asymmetrical than previously.
The border of the summarized outer areas with the so-called extra-areal points of symmetry (Text-fig. 4.6.). The disposition of the points of symmetry following its origin between the rotation areas is the following.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 – 2 | 3 | 7 | | | | | | | | |
| 2 – 3 | | 4 | 4 | | | | | | | |
| 3 – 4 | | 1 | 3 | 6 | | | | | | |
| 4 – 5 | | | | 4 | 6 | | | | | |
| 5 – 6 | | | | | 2 | 5 | | | | |
| 6 – 7 | | | | | | 4 | 3 | | | |
| 7 – 8 | | | | | | | 6 | 6 | 1 | |
| 8 – 9 | | | | | | | | 5 | 3 | 3 |
| 9 – 10 | | | | | | | | 1 | 3 | 6 |
| 10 – 1 | 6 | | | | | | | | 1 | 7 |

### Discussion and Conclusions

1. The results of the newly elaborated and applied two-dimensional rotation methods support the complexity of the biopolymer structure of the sporoderm.
2. On the basis of the first data, the rotation areas may be useful in the investigation of highly organized biopolymer structures. These areas may be highly organized units of the sporopollenin.
3. The highly organized biopolymer structures may have taxonomic and/or phylogenetic significance.
4. The quasi-crystalloid skeleton and the stabilizing biopolymer system are the structural and probably universal units of the plant cell wall.
5. There is still a lot of work to do, our knowledge is not sufficient in this field yet.
6. Finally, for the first attempt a peculiar biopolymer structure was chosen. This one and such fragments will also be used later because one part of this fragment is a quasi-crystalloid with "normal" and peculiar basic units, and another is quasi-

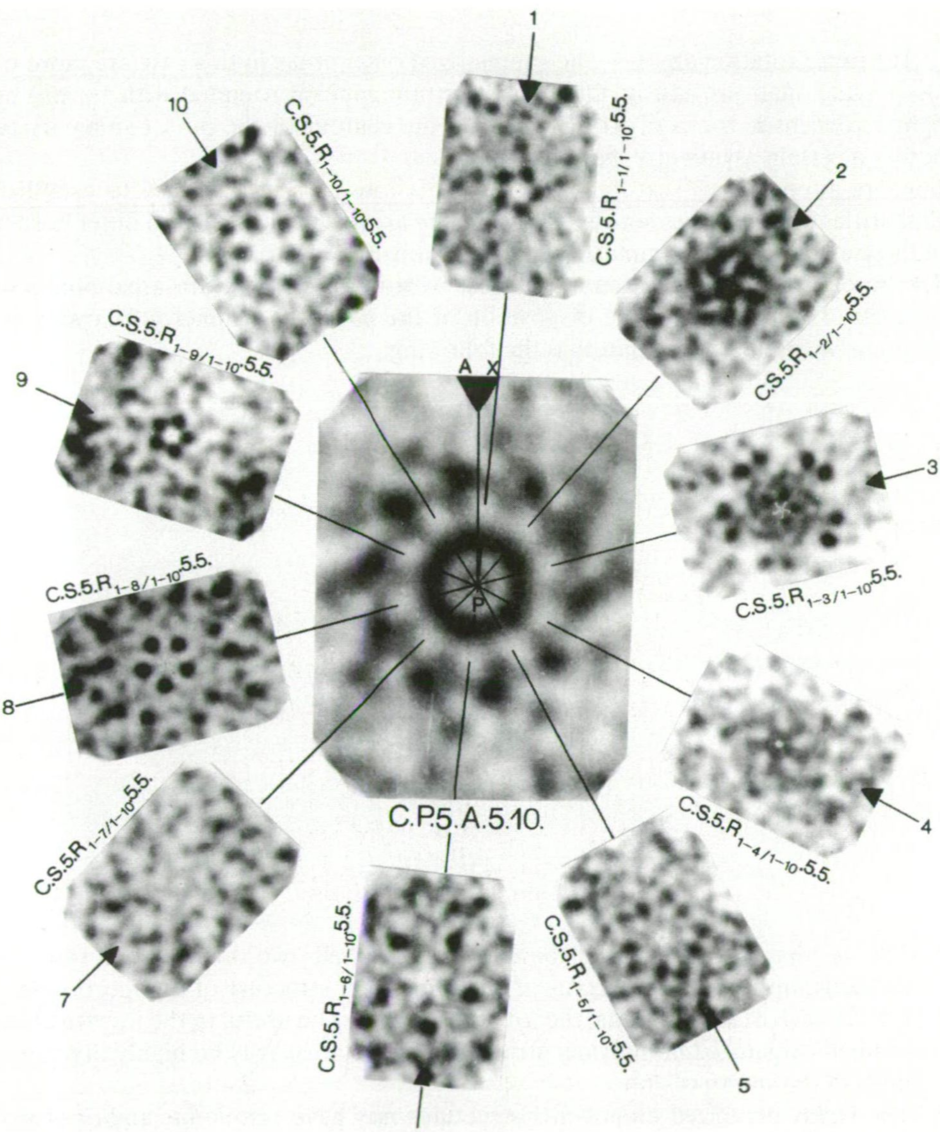
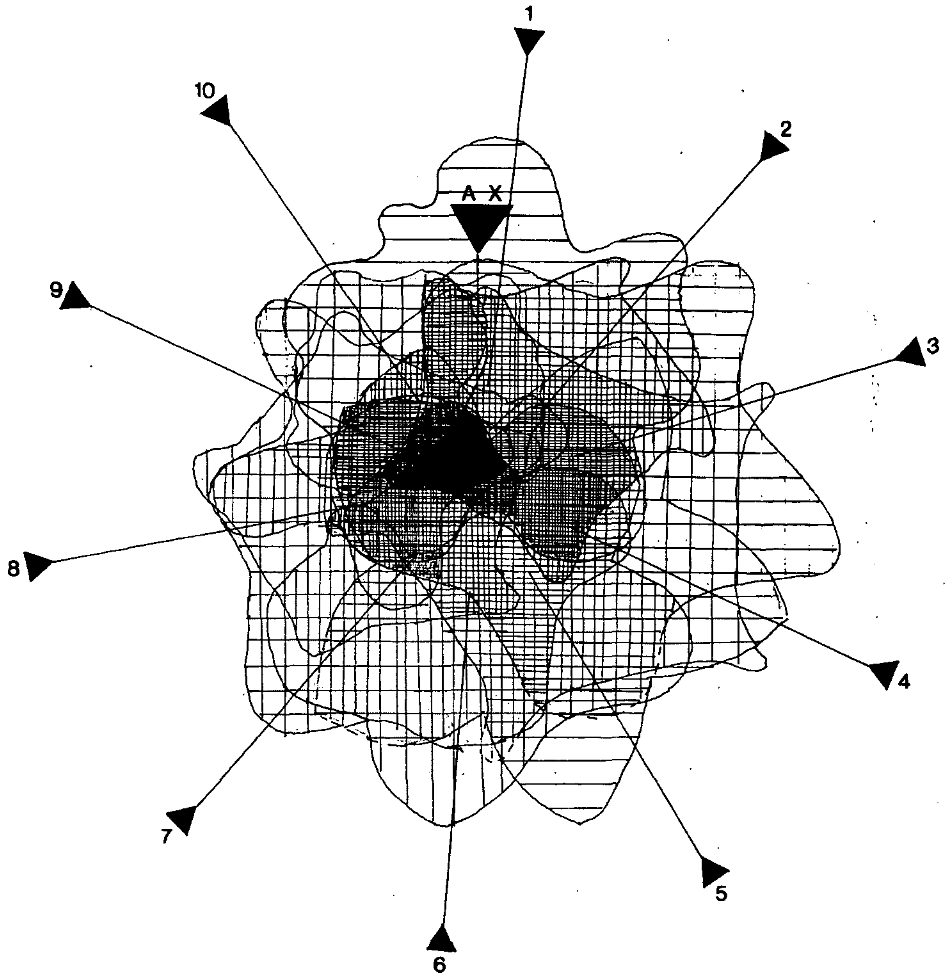


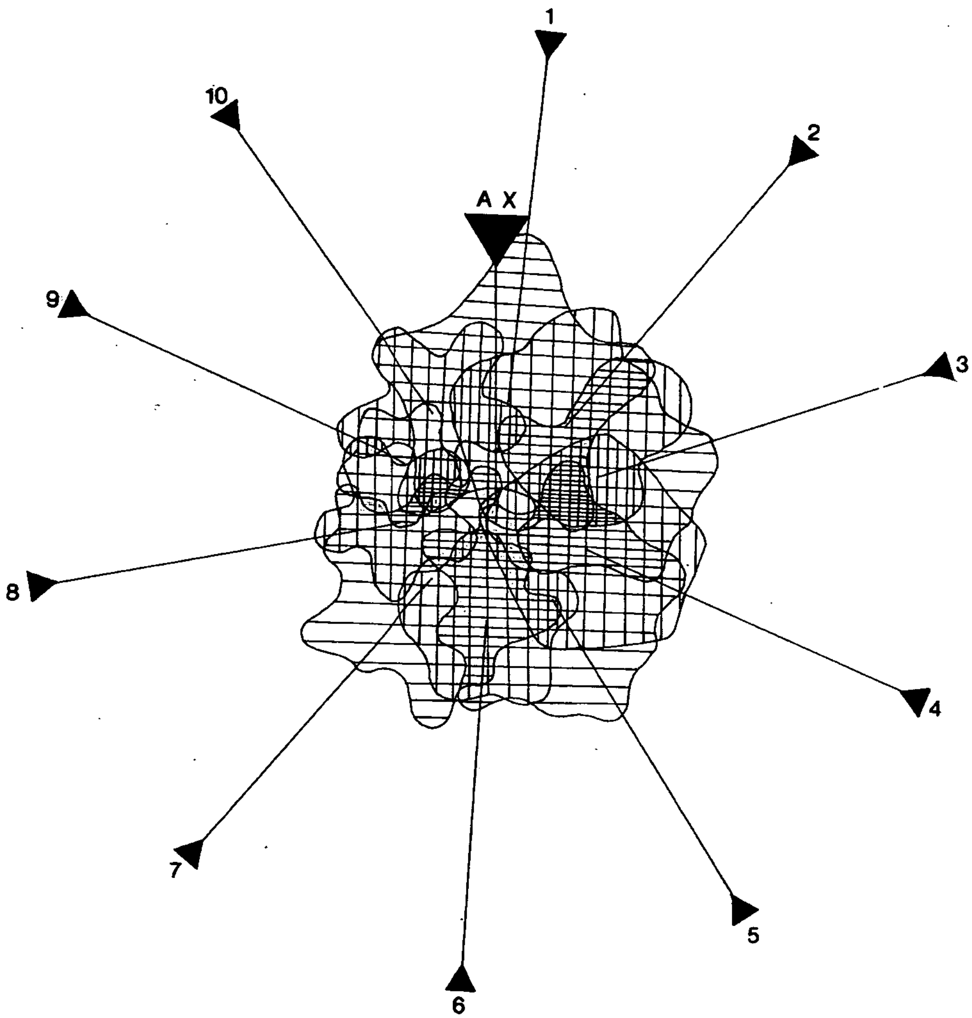

Plate 4.3.

The tenfold rotation picture of the basic biopolymer unit no V as a basis for negative radial rotation. The ten centres of rotation are the light (negative) points of symmetry of the first circle around the centre of the basic biopolymer unit. The results of the radial rotations are oriented in the axes of the radial rotations. $R_{1\,-1/1-10} - R_{1\,-10/1-10}$



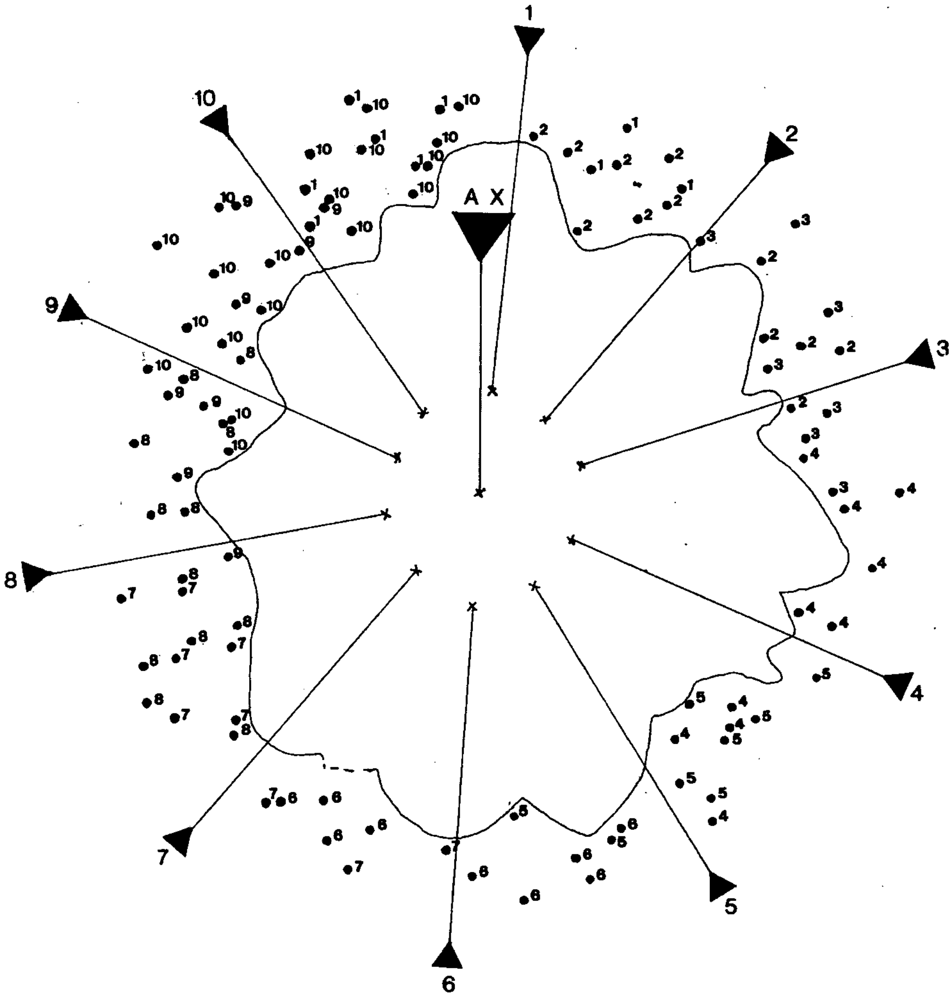

Text-fig. 4.4.

The outer rotation areas of the ten negative radial rotations, cf. Plate 4.3.




Text-fig. 4.5.

The inner rotation areas of the ten negative radial rotations cf. Plate 4.3. In this case it is not possible to establish a periodicity or relationships between the opposite areas.




Text-fig. 4.6.

The summarized outer border of the negative rotation areas with axes of rotations, and the extra-areal points of symmetry. The numbers of the extra-areal points of symmetry indicate the "origin" of these points of symmetry, e. g.: the number of radial rotations.

equivalent with a highly organized structure. The relations between these two kinds of biopolymer systems will be investigated later.

7. These two kinds of biopolymers are probably present in further cell walls, as well, we have found them in the pollen grains of *angiosperms.* (Cf. KEDVES and ROJIK, 1994).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### References


GLIKSON, M., LINDSAY, K. and SAXBY, J. (1989): *Botryococcus* – A planktonic green alga, the source of petroleum through the ages: Transmission electron microscopical studies of oil shales and petroleum source rocks. – Org. Geochem. *14*, 595–608.

KEDVES, M. (1983): Étude paléobotanique sur les schistes pétrolifères du Tertiaire supérieur de Hongrie. – Rev. de Micropaléontologie *26*, 48–53.

KEDVES, M. (1986): Dégradation expérimentale des colonies du genre *Botryococcus* des schistes pétrolifères du Tertiaire supérieur de Hongrie. – Acta Biol. Szeged. *32*, 39–48.

KEDVES, M. (1988): About the symmetry of the pentagonal basic biopolymer units of the pollen wall. – Acta Biol. Szeged. *34*, 157–159.

KEDVES, M. (1989): Méthode d'étude des biopolymères de la paroi pollinique à structure quasi-cristalloïde. A method of investigation of the quasi-crystalloid structure of pollen wall biopolymers. – Rev. de Micropaléontologie *32*, 226–234.

KEDVES, M. (1990): Quasi-crystalloid basic molecular structure of the sporoderm. – Rev. Palaeobot. Palynol. *64*, 181–186.

KEDVES, M. (1991a): TICOS polyhedra as a model in the pentasporan organization. – Plant Cell Biology and Development (Szeged) *2*, 63–74.

KEDVES, M. (1991b): Three dimensional modelling of the biopolymer structure of the plant cell wall I. – Plant Cell Biology and Development (Szeged) *2*, 63–74.

KEDVES, M. (1992): Three dimensional modelling of the biopolymer structure of the plant cell wall II. – Plant Cell Biology and Development (Szeged) *3*, 67–87.

KEDVES, M. and FARKAS, E. (1991): Basis of the tertiary rotation and TICOS modelling of the quasi-crystalloid biopolymer skeleton of the plant cell. – Plant Cell Biology and Development (Szeged) *2*, 36–42.

KEDVES, M. and KEDVES, L. (1995): Computer modelling of the quasi-crystalloid biopolymer structures I. – Plant Cell Biology and Development (Szeged) *6*, 68–77.

KEDVES, M., PÁRDUTZ, Á., FARKAS, E. and VÉR, A. (1991): Basic establishments of the biological objects molecular structure containing quasi-crystalloid skeleton. – Plant Cell Biology and Development (Szeged) *1*, 35–37.

KEDVES, M. and ROJIK, I. (1994): Buckminsterfullerene-like biopolymer units from the exine of *Thalictrum flavum* L. – Plant Cell Biology and Development (Szeged) *5*, 58–66.

KEDVES, M., ROJIK, I. and VÉR, A. (1991): Biopolymer organization of the partially degraded oil shale with the fragmentation method. – Plant Cell Biology and Development (Szeged) *1*, 28–31.

KEDVES, M., ROJIK, I. and VÉR, A. (1992): Ultrastructure and biopolymer organization of the *Botryococcus* colonies from Hungarian alginite. – Workshop on Pyrolysis in Organic Geochemistry. International Workshop, Abstracts, 21–22.

KEDVES, M., TÓTH, A. and FARKAS, E. (1992): Experimental investigation of the biopolymer organization of the sporoderm (recent and fossil). – 8th Internat. Palynol. Congress, Aix-en-Provence, Abstracts, 75.

KEDVES, M., TÓTH, A. and FARKAS, E. (1993): An experimental investigation of the biopolymer organization of both recent and fossil sporoderm. – Grana Suppl. *1,* 40–48.

VÉR, A. (1994): LM investigations of different stained fossil *Botryococcus* colonies. – Plant Cell Biology and Development (Szeged) *5,* 11–19.